## REMARKS

Applicants' representative gratefully acknowledges the withdrawal of the rejections under 35 U.S.C. § 112, second paragraph and 35 U.S.C. § 102(b) in the Final Office Action sent November 1, 2007. The claims remain rejected under 35 U.S.C. § 103(a). Applicants' arguments addressed to that ground of rejection are set forth below.

**Rejection under 35 U.S.C. § 103(a)**

Claims 1-6 and 8-9 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Sode, et al. (WO 2002/36779), in view of Herbaud, et al. (BBA 2000, 1481 (1): 18) as evidenced by Arslan, et al. (BBRC, 1998, 251: 744).

Applicants' claimed invention is directed to co-expression of the α and β subunit of glucose dehydrogenase (GDH) of *Burkhorderia cepacia* (and optionally the γ subunit, claim 3) in combination with genes of a ccm operon. Applicants provide further arguments that the GDH activity obtained with the claimed invention was unexpectedly high which could not have been predicted based upon the combination of references.

The Office Action states that

> ...all of the claimed elements were known in the prior art and one skilled in the art could have combined the element as claimed by known methods with no change in their respective functions, and the combination would have yielded predictable results to one of ordinary skill in the art at the time of the invention. Each of the elements (α- and β- subunit of GDH; ccm Operon; and [sic]) are taught by Sode in view of Herbaud et al. and as evidenced by Arslan et al. It would be therefore predictably obvious to use a combination of these three elements in a recombinant Escherichia bacterium. (Office Action, page 5, lines 7-14).

In response, it was not predictable at the time of the claimed invention that the presence of the ccm operon would result in an increased level of cytochrome C as indicated by Arslan, et al. who teach that inclusion of ccm with cytochrome c550 of *B. subtilis* did not produce any increase in production of cytochrome c (see page 747, col. 1, last paragraph). They conclude that low levels of ccm gene products must be present already and that addition of ccm genes (pEC86) therefore produced no stimulation.

Although Arslan, et al. teach instances where cytochrome maturation genes (ccm) increased production of both endogenous and foreign c-type cytochromes (see Abstract), in at

least one instance stimulation of cytochrome c production by expression of ccm genes was not observed as inclusion of ccm with cytochrome c550 of *B. subtilis* did not produce any increase in production (see page 747, col. 1, last paragraph).

Furthermore, neither Arslan, et al. nor Herbaud, et al. teach or suggest that expression of a ccm system in E. coli has any effect on a glucose dehydrogenase (or other enzyme) activity. Both Herbaud, et al. and Arslan, et al. merely indicate that, at least in some cases, expression of the ccm genes facilitates production of mature cytochrome c. In contrast, Applicants report a 23 fold increase (32 U/mL vs. 1.4 U/mL) in GDH activity in the presence of the ccm system in *E. coli* versus production in *Burkhorderia cepacia* KS1 strain (present specification, page 20, second full paragraph). This increase in GDH activity could not have been predicted based upon the disclosure of Herbaud, et al. and Arslan, et al. on stimulation of cytochrome C levels, especially as the stimulation in maturation of cytochrome c was not observed in all cases as discussed above.

Additionally, Applicants argue that unexpected results were obtained with the claimed combination compared to the prior art. As stated above, Applicants report a 23 fold increase (32 U/mL vs. 1.4 U/mL) in GDH activity in the presence of the ccm system in *E. coli* versus production in *Burkhorderia cepacia* KS1 strain (present specification, page 20, second full paragraph). By co-expressing the enzyme complex including the γ-subunit, the α-subunit and the β-subunit with the ccm genes, the GDH activity in *Escherichia* bacterium increased to a level that was unexpected (present specification, page 20, second and third full paragraphs) compared to expressing the enzyme complex only and the wild type strain. While the activity of the recombinant *E. coli* which also included the genes for the ccm operon (JM109/pTRC99Aγαβ, pBBJMccm) was 32 U/mL, the two controls had activities of only 0.3 (JM109/pTRC99Aγαβ) and 1.4 (*Burkhorderia cepacia* KS1). Such high expression levels could not have been predicted from the cited references.

The Office Action states that the references "also suggest unexpectedly levels of GDH" (Office Action, page 5, 6th line from bottom). However, as discussed above, Herbaud, et al. and Arslan, et al. teach effects on cytochrome C levels, not GDH activity. Furthermore, Herbaud, et al. report that the highest amounts of cytochrome c produced were on the order of 300 μg/L of culture when 0.1 mM δ-aminolevulinic acid was included along with the ccm system under

aerobic conditions (page 22, col. 1, first partial paragraph) which is about the same as what could be produced in *D. vulgaris* (page 22, col. 1, first partial paragraph). Accordingly, Herbaud, et al. do not show levels of cytochrome C production that are unexpected and do not show any effect on activity of an enzyme such as GDH.

Sode, et al. teach the β subunit of GDH but does not suggest a method combining GDH with ccm.

In view of Applicants' arguments, reconsideration and withdrawal of the above ground of rejection is respectfully requested.

**No Disclaimers or Disavowals**

Although the present communication may include characterizations of claim scope or referenced art, the Applicants are not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. The Applicants reserve the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that the Applicants have made any disclaimers or disavowals of any subject matter supported by the present application.

**Co-Pending Applications of Assignee**

Applicant wishes to draw to the Examiner's attention to the following co-pending applications of the present application's assignee. Application in **bold** corresponds to the above-referenced application.

| Serial Number | Title | Filed |
|---|---|---|
| 09/817,251 | METHOD FOR STIRRING LIQUIDS | 03/27/01 |
| 10/466,453 | QUANTITATIVE ANALYZING METHOD AND QUANTITATIVE ANALYZER USING SENSOR | 12/02/03 |
| 10/481,397 | INFORMATION COMMUNICATION SYSTEM | 12/19/03 |
| 10/483,205 | ADJUSTABLE LANCING DEVICE | 01/07/04 |
| 10/493,919 | TEST APPARATUS | 04/27/04 |

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|---|---|---|
| 10/862,465 | METHOD AND IMPLEMENT FOR OPENING HOLE IN SOFT MATERIAL | 06/08/04 |
| 10/498,782 | SAMPLE MEASURING DEVICE | 06/10/04 |
| 10/533,601 | ANALYTICAL TOOL | 04/29/05 |
| 10/545,852 | METHOD OF DETECTING CHLAMYDIA TRACHOMATIS AND KIT THEREFOR | 08/17/05 |
| 10/547,354 | DNA AMPLIFICATION METHOD AND KIT THEREFOR | 08/29/05 |
| 11/220,622 | SUPPLEMENT FOOD FOR LOW BLOOD GLUCOSE RECOVERY | 09/08/05 |
| 10/553,576 | METHOD OF DETECTING OR QUANTITATIVELY DETERMINING MITOCHONDRIAL DNA 3243 VARIATION, AND KIT THEREFOR | 10/17/05 |
| 10/536,822 | METHOD AND APPARATUS FOR CONCENTRATION AND PURIFICATION OF NUCLEIC ACID | 10/18/05 |
| 10/553,509 | METHOD OF DETECTING B3 ADRENALINE RECEPTOR MUTANT GENE AND NUCLEIC ACID PROBE AND KIT THEREFOR | 10/18/05 |
| 10/553,614 | METHOD OF DETECTING PANCREATIC ISLET AMYLOID PROTEIN MUTANT GENE AND NUCLEIC ACID PROBE AND KIT THEREFOR | 10/18/05 |
| 10/553,376 | METHOD OF ISOLATING NUCLEIC ACIDS, AND KIT AND APPARATUS FOR NUCLEIC ACID ISOLATION | 10/19/05 |
| 10/536,829 | DEVICE FOR PRETREATING SPECIMEN | 10/31/05 |
| **10/550,671** | **PROCESS FOR PRODUCING GLUCOSE DEHYDROGENASE** | **11/09/05** |
| 11/587,333 | MUTANT GLUCOSE DEHYDROGENASE | 10/19/06 |
| 11/712,307 | METHOD FOR DETECTING TARGET NUCLEIC ACID | 02/27/07 |
| 11/665,296 | MUTANT GLUCOSE DEHYDROGENASE | 04/13/07 |

**CONCLUSION**

In view of the foregoing Remarks, it is respectfully submitted that the present application is in condition for allowance. Should the Examiner have any remaining concerns which might prevent the prompt allowance of the application, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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Dated: Jul. 29, 2008

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